AN ANALYSIS OF THE SPAWNING HABITS AND SPAWNING STIMULI OF *CUMINGIA TELLINOIDES*.[1]

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Since the discovery of a slight lunar periodicity in the spawning of *Chætopleura apiculata*, the writer has studied the spawning seasons of a considerable number of animals in the hope of finding other more clearly marked cases. The conclusion of this study is that the phenomenon is of rare occurrence at Woods Hole. *Cumingia tellinoides* is the one species studied critically to date that shows a lunar periodicity. Three other species whose study has been completed show absolutely no periodicity. Others being studied require further investigation before they can be passed upon with any degree of certainty. If semi-scientific reports are trustworthy, this type of periodicity is much more common in the tropics, since several fairly well authenticated cases are reported from the Tortugas and elsewhere.

A part of the data presented in this paper was read before the American Society of Zoölogists at their annual meeting in December, 1922. Since that time the study has been continued and the results are finally presented for publication in their completed form.

SECTION I.

The Spawning Season.

The Duration and Characteristics of the Spawning Season.

Cumingia begins to spawn about the middle of June and continues with variations of activity until the middle of September and sometimes until the first of October. During 1926 the first eggs were obtained on June 21 and the last ones on September 21. The heaviest spawning occurs during the last

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week in June, July and the first half of August, as stated by Morgan (*Jour. Exp. Zoöl.*, 1910). The production of eggs by each female is continuous and covers the entire period. If *Cumingia* which have spawned vigorously in the laboratory are returned to their normal habitat for three weeks or a month, they will again set free large quantities of eggs or sperm showing that the supply is replenished.

Experiment No. 1.—July 7, 1924. A stake was driven off Ram Island and eighteen *Cumingia* were planted at its base. All these had just spawned in abundance. On August 7, seven of these *Cumingia* were again brought to the laboratory and treated as usual to induce spawning. After forty minutes two females had spawned heavily and three males had shed sperm abundantly. One other female spawned after one and one half hours. One of the seven did not spawn. The eggs of all these were fertilized and grew into normal veligers.

Experiment No. 2.—July 6, 1926. Eighteen *Cumingia* that had just spawned heavily were planted off Ram Island. On August 1, nine of these were again brought to the laboratory. Three females spawned actively, three males shed sperm in quantity, three did not spawn.

These two experiments indicate that *Cumingia* spawns more than once in a season and that the production of eggs and sperm is continuous.

There are indications that each female spawns two or three times during the season when left in the natural habitat. This inference is founded upon the facts that the eggs are constantly produced and that spawning seems to be most common at full moon and the days following full moon. Since there is no direct evidence on this point, I do not care to insist upon it, except to refer to the schedule of spawning experiments. (See tables, Section III. on lunar periodicity.) There are clearly marked variations in spawning activity which appear to be associated with phases of the lunar cycle.

Orton 15 has advocated the theory that the duration of the spawning seasons of animals is determined by temperature. According to his conception, spawning by any summer breeding species begins in the spring when the water reaches a certain temperature and ends in the fall when the water drops below that temperature. It may be in order to say that this theory is true only in a very general sense, since the species that have been studied by me do not agree among themselves in this regard. Thus *Cumingia* began to spawn on June 21, 1926, when the temperature of the water was 60° F. and quit about September 25 when the temperature was 65° F. *Bugula flabella at* began spawning on June 20 in 1926 when the temperature was 60° F. and continued until November 26, when the temperature was 47° F. *Bugula* usually begins to shed its larvæ by June 10 and continues until late in November, which makes the discrepancy in temperature less pronounced, June 10 temperature being approximately 55° F., November 25 temperature 45° F. *Chætopleura* begins to spawn about June 25, temperature 60° to 65° F. and ends early in September, temperature 65° to 70° F.

Spawning is caused at particular times in nature by various specific stimuli and it is not determined by temperature alone.

Orton gives convincing evidence to show that temperature is the chief factor in determining the limits of the spawning seasons of marine invertebrates. He, however, dismisses the factor light with too little emphasis. Although most tropical animals may spawn throughout the long summer in accordance with his theory, the Palolo worm spawns on one or two days only; and the ripening of its gametes is influenced by light according to several investigators. Light plays a part both in the maturing of the gametes and in spawning in several well-known cases. A very striking example is *Dictyota* at Beaufort, N. C., as described by Hoyt. Temperature, no doubt, is the chief factor in determining the spawning season just as it is also the most important factor in growth and all protoplasmic activity.

SECTION II.

Artificial Spawning Stimuli.

I. Shock.

As stated in my paper on *Chætopleura* (Biol. Bull., 1922), certain marine invertebrate animals will spawn when placed in an artificial or unusual environment, stimulated apparently by shock. As a case in point, if the tube forming annelid Hydroides is removed from its shell and the exposed worm placed in a dish of sea water it will spawn at once, the eggs or sperm seeming to come from every nephridium of the body. This worm will always spawn under these circumstances whether the gametes are fully mature or not. Half-grown eggs are as readily spawned as mature ones. Hydroides will spawn in the spring before any of the eggs are even approximately mature.

In like manner, *Cumingia* spawn readily in the laboratory. It is only necessary to place them in a bowl of sea water and allow them to remain undisturbed for half an hour or forty-five minutes. They usually begin to spawn in half an hour. The gametes are expelled through the dorsal siphon which is extended to a great length. As the eggs or sperm accumulate in the siphon they are thrown out forcibly by sudden whip-like contractions of these organs. During the height of the breeding season it frequently happens that every individual brought to the laboratory spawns. It is not to be supposed that all would have spawned on that particular day if left in their natural environment. There is then something about the treatment which involves digging them from the sand and subjecting them to various unusual disturbances which stimulates spawning. It is safe to conclude that this spawning stimulus is shock as in the case of Hydroides. Spawning takes place just the same when sea water is allowed to flow gently through the dish so that the accumulation of CO_2 is not a factor. It is to be noted that shock is an abnormal stimulus. It may be said again that laboratory experiments are frequently unreliable guides to natural behavior and may lead to misinterpretation if depended upon too implicitly.

Chemical Stimuli.

The question next arises: do the sexes stimulate each other in any way? The question as to whether the females are stimulated to spawn by the presence of the male and vice versa has frequently been discussed in connection with various species. Some very clear cases of the existence of such a chemical stimulus have been discovered. This, for instance, is the case in *Nereis limbata* as described by Lillie and Just. When placed together in a dish of sea water both sexes are excited at once to remarkable activity, and while swimming excitedly they expel their sexual products with violent contractions. Galtsoff has recently shown that the female oyster will spawn if living oyster sperm is added to the water. This is opposed to the published statements by Nelson who has thought that the oyster spawns on particularly warm days and that general spawning on oyster beds is induced by temperature. I am under the impression that he is correct in this statement but the implied conclusion that temperature is the only spawning stimulus may be misleading. He shows, however, that spawning occurs on the peaks of rising temperatures which indicates that changes in temperature upward cause males to shed and hence general spawning by all nearby oysters results. Spawning by oysters may take place at any temperature between 68° and 85° F. (Churchill and Nelson).

It was found in the case of *Cumingia* that there is no perceptible stimulus from the opposite sex. They appear to spawn quite as readily when isolated as when in the same dish. Experience has shown that the best way to obtain clean eggs in convenient form for study is to wash the animals free from debris and isolate them in small stender dishes half filled with sea water, or enough water to cover the animal. Both males and females will shed their gametes when so isolated. The eggs are thus obtained free from sperm and may be artificially fertilized at will. Drew was the first to use this method and he was under the impression that drying accentuates or constitutes the spawning stimulus. Morgan in 1910 noted that *Cumingia* will spawn when isolated (*Jour. Exp. Zoöl.*, Vol. 9, p. 595). He thinks, however, that the presence of spermatozoa in the water may incite the females to spawn more promptly than they otherwise would. The stimulus in this case, if authenic, might be either chemical or physical. One is likely to gain the same impression by watching the spawning of *Chætopleura* (*Chiton*) because when males and females are placed together in a dish of sea water, the males always shed their products first, and are followed promptly by the females. So far as could be learned, however, spawning by the females occurred as promptly without spermatozoa as with them.

It is the writer's belief that the spermatozoa have no effect. The mechanical shock of removing the animals from their normal situations furnishes all the stimulus that is required to induce spawning. There is no perceptible stimulus from the opposite sex either chemical or physical. This is shown by a series of experiments designed to test the theory. The three described herewith are representative. The method was to collect *Cumingia* in large numbers. Half of them were isolated in small stender dishes and covered with sea water in the usual way. The other half were treated in the same way except that they were all put in a large crystallization dish so that they might receive chemical stimuli from each other if such exist. The time of spawning is shown in the two columns of the tables and they may be readily compared.

Experiment No. I.

August 21. Collected 20 *Cumingia*. Ten were isolated in stender dishes and ten were placed in a common dish. Experiment set at 12:30 P.M.

10 Isolated Individuals Spawned as Follows.	Time Elapsed before Spawning.	10 in Common Dish Spawned as Follows.
1:15 P.M. one female	45 minutes	
1:16 P.M. one male	46 "	
1:20 P.M. one male	50 "	
1:23 P.M. one male	53 "	
	55 "	One male shed 1:25 P.M.
	57 "	One male " 1:27 P.M.
1:29 P.M. one female	59 "	
	60 "	One male " 1:30 P.M.
	61 "	One female " 1:31 P.M.
	67 "	One male " 1:37 P.M.
	68 "	One male " 1:38 P.M.
	70 "	One male " 1:40 P.M.
	71 "	One female " 1:41 P.M.
1:55 P.M. one female	85 "	
2:05 P.M. one female	95 "	

Comment: Three of the isolated individuals and two in the common dish failed to spawn. Comparison shows that those in the common dish did not spawn more promptly than the isolated individuals. It is noteworthy that those in the common dish spawned close together in point of time. This was also true in Experiment No. 3, but not in No. 2.

Experiment No. 2.

August 27. Collected 34 *Cumingia*. Isolated seventeen in stender dishes and placed seventeen in a common dish. Experiment set at 5:55 P.M.

17 Isolated Individuals Spawned as Follows.	Time Elapsed before Spawning.	17 in Common Dish Spawned as Follows.
	30 minutes	One female at 6:25 P.M.
One male at 6:27 P.M.	32 "	
One male at 6:28 P.M.	33 "	
One male at 6:29 P.M.	34 "	
	35 "	Two males at 6:30 P.M.
	36 "	One male at 6:31 P.M.
One female at 6:32 P.M.	37 "	
	38 "	One male at 6:33 P.M.
	39 "	One male at 6:34 P.M.
	41 "	One female at 6:36 P.M.
One male at 6:37 P.M.	42 "	One male at 6:37 P.M.
One female at 6:37 P.M.	42 "	
One male at 6:39 P.M.	44 "	
	47 "	One male at 6:42 P.M.
	48 "	One male at 6:43 P.M.
One male at 6:44 P.M.	49 "	
One female at 6:45 P.M.	50 "	
Two males at 6:46 P.M.	51 "	One female at 6:46 P.M.
	52 "	One female at 6:47 P.M.
	55 "	One male at 6:50 P.M.
One female at 6:51 P.M.	56 "	
One female at 6:54 P.M.	59 "	

Comment: Five isolated individuals did not spawn within the hour and five of those in the common dish did not spawn. There is no evidence that they receive a chemical stimulus from each other calculated to induce spawning. They spawn as readily when isolated as when in the same water.

Experiment No. 3.

August 28. Collected 24 *Cumingia*. Isolated twelve in stender dishes and placed twelve in a common dish. Experiment set at 1:30 P.M.

12 Isolated Individuals Spawned as Follows.	Time Elapsed before Spawning.	12 Individuals in Common Dish Spawned as Follows.
	2 minutes	One male at 1:32 P.M.
1:48 P.M. one male	18 "	
	25 "	One male at 1:55 P.M.
	27 "	One male at 1:57 P.M.
1:58 P.M. one male	28 "	One male at 1:58 P.M.
1:59 P.M. one male	29 "	One male at 1:59 P.M.
	30 "	One female at 2:00 P.M.
	35 "	One male at 2:05 P.M.
	38 "	One male at 2:08 P.M.
	39 "	One male at 2:09 P.M.
2:10 P.M. one female	40 "	
2:13 P.M. one female	43 "	
2:15 P.M. one male	45 "	
2:16 P.M. one male	46 "	
2:17 P.M. one female	47 "	
2:20 P.M. one female	50 "	
2:22 P.M. one female	52 "	

Comment: Two of the isolated individuals and three in the common dish did not spawn within an hour. As a matter of chance most of the females got into the isolated dishes.

SECTION III.

Natural Spawning Stimuli.

I. Lunar Periodicity.

If *Cumingia* spawns most frequently at the period of the full moon, it is evident that there is some cosmic stimulus which varies with the moon's phases to which spawning is due. The two chief variable quantities are light, and tides, or pressure. There is at present no scientific explanation of lunar periodicity although numerous cases are known.

It has been the writer's chief interest during the past five years to learn whether the phenomenon is rare or of common occurrence among animals at Woods Hole. The data bearing on this subject are given in a short forthcoming paper, but the findings in reference to *Cumingia* are given here in greater detail.

A careful study and comparison of data collected during the past five years shows that there is a lunar periodicity in spawning, although it is not as well marked as in *Nereis limbata* or the Suez sea urchin studied by Fox.

There is no clock-like regularity in the spawning of *Cumingia* in nature. Not all individuals spawn promptly at the full moon. Spawning in fact covers a period of two or three weeks in each month. We know this by the quantities of eggs spawned in the laboratory by any particular lot of sexually mature adults brought in. Some set free a maximum quantity, some a small quantity and some only a few eggs or none at all. The last are considered to have spawned recently. By this indirect reasoning, one learns that there is no one particular day on which general spawning occurs. It is likely that spawning occurs when the gonads and their ducts become filled to capacity and this internal pressure no doubt constitutes a second natural spawning stimulus. If the production of gametes were continuous, and uniform in rate, spawning might occur on any day in the month except for this cosmic stimulus which brings about a more or less marked rhythm. I am of the opinion that there is a rhythm in the rate of production of the gametes as well as in the spawning of them. In any case, the only time in the month during which most of the gonads seem empty and during which spawning stimuli seem to be suppressed is the period of the first quarter. Beginning at full moon the heavy spawning is sometimes completed before new moon but more frequently not until near first quarter. In many respects *Cumingia* resembles *Toxopneustes*, the Beaufort sea urchin, whose periodicity was described by Tennent. The production of gametes in this species is rapid and the gonads are soon replenished after spawning occurs.

It should be noted here that there is a distinction between stimuli which bring about the act of spawning and those which bring about the maturing of the gametes. The latter may be an evenly continuous process or it may be enhanced at certain times. The former is merely the act of extruding these gametes and may be induced by some external stimulus, such as those due to the moon in its various phases, or to any other external stimulus.

In the following paragraphs I give the history of this study of *Cumingia* as constituting an important part of the experimental basis for the conclusions drawn.

During the greater part of the breeding season of *Cumingia*, no lunar periodicity in spawning is likely to be observed unless attention is directed to it. *Cumingia* has been used as a source of embryological material for class work for many years and only rarely has difficulty been experienced in obtaining eggs in abundance at any time that they have been needed. It appeared that eggs could be obtained at any time during the summer. It was only after receiving a suggestion from Heilbrunn that any convincing evidence of periodic spawning was obtained. He said that *Cumingia* would spawn a second time after its season had apparently ended in August. Morgan gives the spawning season of *Cumingia* as June, July, and August, so that he evidently overlooked the September spawning after the break in August.

It was at Heilbrunn's suggestion that I undertook experiments late in the summer of 1922, when, as he said, a break could be expected.

During the week of August 23 to September 1, 1922, no eggs could be obtained from *Cumingia*, although the characteristic spawning reactions were carried out as usual. The siphons were extended to great length and the whip-like lashing of these organs was carried out. All that was expelled, however, was a considerable amount of mucus containing at best a few immature or defective eggs. There were no mature eggs in the ovaries.

Bean, working in the same laboratory, was likewise unable to obtain eggs during this period for experimental purposes. Bean worked constantly with *Cumingia* during the summer of 1922 and he thought that he detected a periodicity during the height of the breeding season. The maximum spawning periods, according to his statement, occur at new and full moon, being therefore bimonthly. This is interesting, if correct, as indicating that the spawning stimulus might be associated with the tides rather than with moonlight. Further study during the summers of 1923, 1924, 1925 and 1926 shows conclusively that the maximum spawning comes at full moon and is not bimonthly.

The table for 1922 gives the exact data as obtained by the writer. It will be seen that spawning activity is revived somewhat before full moon after a period of complete cessation. Furthermore a careful study of the tables for the years 1922, 1924 and 1926 shows that the heaviest spawning occurs at the period of the full moon until new moon, and that the period of the first quarter is the period of restricted spawning. This behavior of *Cumingia* can scarcely be explained on any other ground than as a lunar effect.

There is no adequate way of describing the variations in spawning that are so obvious to the experimenter. However, the tables of data appended to this section, together with the comments written at the time the experiments were performed, must suffice to explain the basis of the conclusions arrived at. Although they are long, there seems to be no way to give the evidence more briefly. I give the data for three years only as representative.

Discussion.

There has been much speculation about the cause of lunar periodicity in spawning, but it has for the most part remained speculation and guessing. It can never be solved except by experiments similar to those devised by Mayer. Neither *Cumingia* nor *Chætopleura* is suitable for a study of the causes which have produced lunar periodicity. In the first place, periodicity is not clear-cut in these species and in the second place they can be observed only under laboratory conditions which have been shown to be unreliable. They are affected by shock whereas some species are apparently not so affected.

A casual survey of known cases of lunar periodicity shows a general similarity in all and it is likely that the underlying causes are the same in all. A study which undertakes to explain these phenomena should include a search for other cases and especially all worms that are known to show a lunar periodicity should be studied in detail for purposes of comparison. However, speculation and comparison can scarcely explain it. There is need for experimental methods and the Palolo worm, and the Suez sea urchin or possibly *Nereis limbata* are among the most favorable species for study. It should be a comparatively simple problem to subject them to artificial light, especially polarized light, in addition to all the light to which they are accustomed, to see if they can be thrown out of tune. The Palolo worm seems to offer the best opportunity because of its definite and predictable time of spawning, so that any change from this time could be regarded as an experimental modification.

Summary.

1. The spawning season of *Cumingia* extends from the middle of June until the middle or end of September. The most active spawning usually includes the last week in June and ends about August 20. Each female spawns more than once and the production of gametes is practically continuous.

2. The spawning is heaviest from full moon to new moon and least at first quarter thus showing a lunar periodicity.

3. *Cumingia* is greatly affected by shock and rough treatment so that sexually mature individuals spawn promptly when brought to the laboratory, after being placed in a bowl of quiet sea water.

4. There are apparently no chemical stimuli by which the sexes stimulate each other to sexual activity as is the case in *Nereis limbata* and the oyster. They spawn as readily when isolated as when in close proximity.

5. It is shown that temperature is not the only factor which determines the duration of the spawning season and periods of spawning.

SPAWNING EXPERIMENTS. 1922.

Cumingia tellinoides.

Table No. I.

Cumingia spawned vigorously during the first half of August but stopped suddenly about August 15. Several investigators were experimenting upon Cumingia eggs at that time and noted this fact. All collections were made at low tide or within an hour of low tide at the convenience of the supply department of the laboratory.

* *September 21, new moon.*

I. 8/23. No eggs spawned. 1 male shed sperm abundantly. 17 did not spawn.

II. 8/24. No eggs and no sperm.

III. 8/27. No eggs spawned. 4 males shed sperm small amount. 14 did not spawn.

* *September 28, first quarter.*

IV. 9/1. 5 females spawned in quantity. 6 males shed sperm, 3 in quantity, 3 in small amount. 7 did not spawn.

Comment.—This revival of spawning after the spawning season had apparently ended is surprising. The quantity of eggs shed is equal to mid-season spawning.

V. 9/2. 4 females spawned in quantity, 2 maximum, 2 one half maximum. 5 males shed sperm, 3 heavy and 2 light. 9 did not spawn.

* *September 5, full moon.*

VI. 9/5. 6 females shed eggs, 4 of them maximum. 5 males shed sperm, 3 abundantly and 2 light. 4 did not spawn.

VII. 9/6. 6 females spawned, 4 maximum, 2 one half maximum. 5 males shed sperm, none maximum, mostly light. 8 did not spawn.

Note.—This cessation or reduction of spawning in August is typical as shown by five years' experience. Spawning is also regularly revived in September. It is most marked when full moon falls during the first week of the month; and less marked when it is near the middle of the month. This record for 1922 led the writer to study these phenomena in detail in succeeding years. They can scarcely be explained on any other ground than a lunar cycle effect.

SPAWNING EXPERIMENTS. 1924.

Cumingia tellinoides.

Table No. II.

* *July 2, new moon.*

I. 7/5. 6 females spawned well (maximum). 5 males shed well (maximum). One did not spawn.

II. 7/8. 4 females spawned well (maximum). 5 males shed well (maximum). 3 did not spawn.

* *July 9, first quarter.*

III. 7/9. 100 per cent. spawned. 3 females spawned, 2 heavy, one light. 4 males shed, 2 heavy, 2 light.

Remark.—Heavy spawning activity during the past week and nearly 90 per cent. of the individuals spawning approximately maximum quantity.

IV. 7/12. 1 female spawned small amount 1/2 maximum. 4 males spawned, 1/2 maximum. 10 did not spawn.

V. 7/14. 6 females spawned, one heavy, 2 medium and 3 light. 9 males spawned, one heavy, 4 medium, 4 light. 10 did not spawn after hours.

Comment.—Light spawning activity compared with ten days ago which was remarkably heavy. Tendency to spawn reluctantly, some individuals spawning only after hours. One spawned after two hours, others slow to act. Only one good lot of eggs from twenty-five individuals, and none on July 12.

* *July 16, full moon.*

VI. 7/19. 4 females shed, 2 maximum, 2 light. 5 males shed, 3 maximum, 2 light. 6 did not spawn.

Comment.—Impression of light or medium spawning activity.

* *July 23, third quarter.*

VII. 7/25. 12 females shed, 5 maximum, 3 medium, 4 light. 7 males shed. Some very heavy, some light. 5 did not spawn.

Comment.—Impression of heavy spawning activity.

VIII. 7/31. 7 females shed, 4 heavy, one medium, 2 very light. 7 males shed, 5 heavy, 2 light. 2 did not spawn.

Comment.—Impression of heavy spawning activity, but not quite as heavy as at last new moon.

* *July 31, new moon.*

IX. 8/2. 5 females shed, 3 heavy, 2 light. 6 males shed, 4 heavy, 2 light. 7 did not spawn.

Comment.—Only one female spawned within an hour. And only two males within the first hour. (Why?) No others spawned for two and one half hours. Impression of poor spawning activity, reluctant and evident retention of eggs from some cause, also low percentage spawned after hours.

* *August 7, first quarter.*

X. 8/12. 11 females shed, 5 near maximum, 2 medium, 4 light. 14 males shed, all heavy or medium. 2 did not spawn within the first hour.

Comment.—This lot began to spawn after fifteen minutes, males more quickly than females. Most of the males began before thirty minutes. Some females began after twenty-five minutes but most of them after 40 minutes. Fourteen males and eight females had shed within one hour. Three females spawned after one and one half hours.

Impression of good heavy spawning activity and the reluctance observed a week ago was gone. The spawning was unusually prompt and vigorous. Cause?

* *August 14, full moon.*

XI. 8/16. 100 per cent. spawned. 6 females shed, 4 heavy, 2 medium. 3 males shed, 2 heavy, one medium. 1 spawned after $1\frac{1}{2}$ hours heavily.

Comment.—Very heavy spawning activity. All except one spawned within an hour. Most of them began in 20 to 30 minutes. The last one spawned heavily after one and one half hours.

Heilbrun collected twelve Cumingia this date. All but one spawned.

XII. 8/20. 6 females spawned within an hour, 4 heavy, 2 medium. 13 males shed, 7 heavy, 3 medium, 3 light. 2 females spawned after $1\frac{1}{2}$ hours. Very few eggs from these two. 1 did not spawn.

* *August 22, third quarter.*

XIII. 8/26. 100 per cent. spawned. 12 females shed during first hour, 5 heavy, 2 medium and 5 a mere trace. 9 males shed, 5 heavy, four light. 2 females spawned after one hour and forty minutes. The last two spawned very few eggs.

Comment.—Impression of heavy spawning activity during the past ten days. Five females gave only a very few eggs which indicates recent spawning on their part at the full moon period.

The inhibition to spawning noticed earlier is not now working. The heaviest spawning of the year has occurred during the past ten days.

XIV. 8/29. None spawned during the first hour. 4 females spawned a few eggs later, 1/10 maximum. 2 males shed a trace of sperm. 14 did not sperm.

XV. 8/30. 2 females spawned. Very few eggs, approximately 1/15 maximum. 3 males shed lightly, 1/10 maximum. 15 did not spawn.

* *September 1, new moon.*

XVI. 9/2. 5 females shed a few eggs, scarcely visible in the dish (a trace). 2 males made water slightly turbid with sperm. 18 did not spawn.

Comment.—Spawning negligible. Experiments for 1924 ended at this time. Experience has shown that spawning no doubt revived at the approach of the September full moon for a few days. This series of experiments shows that spawning was heavy early in July from the time the experiments of the season began until after the first quarter when they suddenly fell off not to be fully revived until somewhat after full moon. Then followed a week of heavy spawning until new moon.

Shortly after the first quarter in August the heaviest spawning of the year began and continued until the approach of the next new moon or from August 7 to August 29. Then came the usual late season, temporary cessation of spawning which presumably revived somewhat at the September full moon.

It is unfortunate that these experiments could not have begun by the middle of June and continued until the middle of September. The experience of several years leads one to believe that spawning began at full moon in June and reached maximum late in June. The variations in spawning activity under laboratory conditions show that the production of the gametes is greatest around full moon and least around the first quarter. These results are shown regularly except at the height of the breeding season when it may be masked by the rapidity of egg production.

SPAWNING EXPERIMENTS. 1926.

Cumingia tellinoides.

Table No. III.

The following table of experiments shows the duration of the spawning season and variations in spawning activity during the lunar cycle. From fifteen to twenty-five individuals were used in each experiment. They were isolated in stender dishes containing enough water to cover them fully. Spawning usually occurs within an hour. Collections were always made at low tide.

I. 3/1. No eggs and no spermatozoa.

II. 4/1. No eggs and no sperm.

III. 6/1. No eggs and no sperm. (Experiment by Hugh Montgomery.)

IV. 6/11. No eggs and no sperm. (Experiment by Hugh Montgomery.)

V. 6/15. No eggs, one male shed active spermatozoa, one fifth maximum.

* *June 18, first quarter.*

VI. 6/21. 2 females spawned, approximately 1/5 maximum. 2 males shed sperm, 1/4 maximum. 8 did not spawn.

Comment.—Eggs mature, cleavage normal. Spawning season opened between June 15 and June 20.

* *June 25, full moon.*

VII. 7/1. 6 females spawned, 1/4 to 1/3 maximum. 7 males shed sperm, 1/3 to 1/2 maximum. 2 did not spawn.

* *July 2, third quarter.*

VIII. 7/6. 9 females spawned rather abundantly, 1/2 to 3/4 maximum. 12 males shed sperm, 11 heavy, 1 light. 2 did not spawn.

* *July 9, new moon.*

IX. 7/15. 2 females spawned, 1/2 to 2/3 maximum. 12 males shed sperm, 10 medium, 2 light. 12 did not spawn.

Comment.—Light spawning, and many failed to spawn.

* *July 17, first quarter.*

X. 7/19. 2 females spawned 1/20 maximum (very few eggs). 4 males shed sperm, very light, 1/10 to 1/20 maximum, scarcely detectable in the dish. 13 did not spawn.

Comment.—Spawning all but extinct. Not even one fair lot of eggs obtained. Scarcely visible in the dish. Almost no spawning activity during the past week.

XI. 7/24. 13 females spawned lightly, 1/2 to 1/3 maximum. 7 males shed sperm, 6 heavy, 1 light. 2 did not spawn.

Comment.—Great revival of spawning but light in amount.

* *July 25, full moon.*

XII. 7/26. 8 females spawned, 4 heavy (maximum) and 4 medium (1/2 to 2/3 maximum). 12 males shed sperm, 6 heavy, 4 medium, and 2 light. 6 did not spawn.

Comment.—Very heavy spawning. The heaviest this year to date. All spawned promptly after thirty or forty minutes. Lunar periodicity is demonstrated clearly. Almost complete cessation of spawning ten days before full moon but came on again actively at full moon.

* *July 31, third quarter.*

XIII. 8/4. All spawned actively, 100 per cent.

* *August 8, new moon.*

XIV. 8/10. 6 females spawned, 3 heavy, 2 medium, 1 light. 11 males shed sperm, 10 heavy or medium, 1 light. 6 did not spawn.

XV. 8/13. 7 females spawned, 6 medium or heavy, 1 light. 7 males shed sperm, mostly heavy. 1 did not spawn.

XVI. 8/16. 100 per cent. spawned. 8 females spawned in quantity. 7 males shed sperm in quantity.

* *August 16, first quarter.*

XVII. 8/20. 5 females spawned (1/2 maximum or less). 7 males shed sperm, 1/2 maximum. 2 did not spawn.

XVIII. 8/21. 6 females spawned heavily, 1/2 to 3/4 maximum. 9 males shed sperm heavily. 5 did not spawn.

* *August 23, full moon.*

Comment.—There is no evidence of reduced spawning at the first quarter in August, whereas in July there was a clear-cut case.

XIX. 8/28. 7 females spawned, approximately 1/2 maximum. 12 males shed sperm, medium to light. 5 did not spawn.

Comment.—Spawning at this time by about 80 per cent. of the individuals but for the most part below the spawning of last week in quantity. Good lots of eggs still obtained, 1/2 to 1/3 maximum the rule. The quantity of eggs spawned by each individual is usually reduced toward the end of the season.

* *August 29, third quarter.*

XX. 8/31. 3 females spawned, 2 1/10 maximum, 1 1/20 maximum. 4 males shed sperm (1/4 maximum). 25 did not spawn.

XXI. 9/1. 6 females spawned 1/20 maximum (scarcely visible in the dish). 4 males shed sperm, very light, 1/10 maximum, water only slightly turbid. 14 did not spawn.

XXII. 9/3. No eggs and no sperm. Some extended the siphons and lashed them in characteristic manner but there were no eggs in the ducts.

Comment.—Very light spawning. Almost none since August 28. Spawning suddenly fell off, one week after full moon. Vigorous spawning continued for five days after full moon.

* *September 7, first quarter.*

XXIII. 9/9. 6 females spawned, 1 2/3 maximum, 4 1/5 maximum, 1 1/10 maximum. 5 males shed sperm, 3 1/2 maximum, 2 light. 19 did not spawn.

Comment.—Considerable revival of spawning noted. Much more active than last week, but still relatively insignificaut. Good for so late in the season.

* *September 21, full moon.*

XXIV. 9/21. 3 females spawned, 1 maximum, 1 1/5 maximum, 1 1/10 maximum. 2 males shed sperm, 1 3/4 maximum, 1 1/2 maximum. 15 did not spawn.

Comment.—Revival of spawning evident, though not extensive. Quantities surprisingly large. Temperature 18° C., or 17½° C.

XXV. 9/10. Temperature 17° C. No eggs and no sperm.

Comment.—The spawning season of Cumingia ends between September 15 and October 1. This year spawning continued until the end of September. The foregoing data give no very definite information about the time of spawning in nature. It is evident that eggs are produced almost continuously although probably not uniformly. There is a lunar periodicity either in the production of the gametes or in the spawning or in both. Spawning by each individual occurs more than once during the season.

BIBLIOGRAPHY.

Mr. H. Munroe Fox gives a fairly complete bibliography of papers that dea with lunar periodicity in his paper entitled "Lunar Periodicity in Reproduction." Proc. Royal Soc., Vol. 95 (1923). It seems unnecessary to include them all here. I select a few that are likely to be of local interest or important to the general reader.

1. **Crozier, W. J.**

'22 An Observation of Cluster Formation of the Sperms of *Chiton*. Am. Nat., Vol. 56, p. 478.

2. **Friedlander, B.**

'98–'04 Ueber den sogennanten Palolowurm. Biol. Centralbl., Vols. 18, 19, 21, and 27.

3. **Fox H. Munroe.**

'22–'23 Lunar Periodicity in Reproduction. Nature, Vol. 109 (1922) and Proceedings of the Royal Soc. B, Vol. 95, p. 523 (1923).

4. **Grave, B. H.**

'22 An Analysis of the Spawning Habits and Spawning Stimuli of *Chætopleura apiculata*. Biol. Bull., Vol. 42.

5. **Hempelmann, F.**

'11 Zur Naturgeschichte von Nereis dumerilii. Zoologica, Vol. 25.

6. **Hoyt, W. D.**

'07 Periodicity in the Production of the Sexual Cells of *Dictyota dichotomi*. Bot. Gaz., Vol. 43.

7. **Just, E. E.**

'14 Breeding Habits of the Heteronereis form of *Platynereis megalops* at Woods Hole, Mass. Biol. Bull., Vol. 27.

8. **Just, E. E.**

'20 The Fertilization Reaction in *Echinarachnis parma*. Biol. Bull., Vol. 30.

9. **Kraemer, A., and Collin, A.**

'97 Ueber den Bau der Korallenriffe, etc., and Ueber den Palolowurm. Kiel. (Lipsius & Fischer.) (See also Biol. Centralbl., 1899.)

10. **Lillie, F. R., and Just, E. E.**

'13 Breeding Habits of the Heteronereis form of *Nereis limbata* at Woods Hole, Mass. Biol. Bull., Vol. 24.

11. **Lewis, I. F.**

'10 Periodicity in Dictyota at Naples. Bot. Gazette, Vol. 50.

12. **Mayer, A. G.**

'10–'02 The Atlantic Palolo. Bull. Mus. Comp. Zoöl., Harvard, Vol. 36 (1900), also Science Bull. Mus. Brooklyn Acad. Arts and Sci. (1902).

13. **Mayer, A. G.**

'08–'09 The Annual Breeding Swarm of the Atlantic Palolo. Carnegie Inst. Wash. Pub. No. 102 (1908); also Proc. 7th Internat. Zoöl. Cong., Boston.

14. **Nelson, T. H.**

'21 Aids to Successful Oyster Culture. N. J. Agr. Exp. Sta. Bull. No. 351.

15. **Orton, J. H.**

'20 Sea Temperature, Breeding and Distribution in Marine Animals. Jour. Mar. Biol. Assoc., New Series, Vol. 12, p. 339.

16. **Scott, W. J.**

'09 Some Egg-laying Habits of *Amphetrite ornata*. Biol. Bull., Vol. 17.

17. **Tennent, D. H.**

'10 Variations in Echinoid Plutei. Jour. Exp. Zoöl., Vol. 9, p. 657.

18. **Mark, E. L.**

(Unpublished manuscript on *Odontoglossus*.)